Inducing Competence (Modified Campylobacter jejuni Protocol):

Materials Needed:
Two TSA plates (Bacterial Lawn Grown)
One empty TSA plate
Wash Buffer (26mM Sucrose, 15% Glycerol)
Centrifuge
1.5mL Eppendorf Tubes
TSB
Pipets

Protocol (estimated time 1 hour):

1. Remove the bacteria from the plates by pipetting 2mL TSB onto the plates, followed by scraping the cells off of the TSA with an inoculation loop.
2. Once the bacteria are scraped off the plate and floating/suspended in the TSB, angle the plate to one side and pipet up all the TSB (contains the cells)
    a. The solution is then dispensed into the 1.5ml eppendorf tubes.
3. Repeat steps 1 and 2 the other plate.
4. Pellet the cells by centrifugation at >10000g for 5 minutes.
5. Remove the supernatant without disturbing the bacterial pellet.
6. Resuspend the bacterial pellet in 1ml ice cold wash buffer.
    a. The pellet can be resuspended by gently pipetting up and down close to the bacterial pellet.
7. Repeat steps four through six, three times.
    a. each time, resuspending the cells will get easier and easier.
8. For the last time, resuspend the bacterial pellet in 400ul of ice cold wash buffer.
9. The cells can immediately be used or can be frozen at -80C in 100ul aliquots.
10. Streak out the competent cells to ensure they survived the washes and no contamination.

Inducing Competence (Modified Salmonella Protocol):

Materials Needed:
Two TSA plates (Bacterial Lawn Grown)
Centrifuge
1.5mL Eppendorf Tubes
50ml Sterile Falcon Tubes
TSB
Pipets
1mM HEPES Buffer (pH:7)
10% Glycerol Solution
Ice Bucket
One Empty TSA Plate

Protocol (estimated time 2 hours):

1. Remove the bacteria from the plates by pipetting 4mL TSB onto the plates, followed by scraping the cells off of the TSA with an inoculation loop.
2. Once the bacteria are scraped off the plate and floating/suspended in the TSB, angle the plate to one side and pipet up all the TSB (contains the cells)
    a. The solution is then dispensed into a 50mL falcon tube.
3. Repeat steps one and two.
    a. However, place the cells into the same 50ml falcon tube as used in step one and two.
4. Chill the cells on ice for 15min
    a. during this time, set the centrifuge to cool down to 4C.
5. Pellet the cells by centrifugation at 2300g for 10min (4C).
    a. Remove the supernatant by pouring it out the top.
        i. Be sure to quickly flame the lid of the falcon tube.
6. Resuspend the cells by gently shaking in 40mL chilled 1mM HEPES buffer.
7. Pellet the cells by centrifugation at 2300g for 10min and remove the supernatant.
8. Repeat steps five and six with 20mL chilled 1mM HEPES buffer, followed by 20mL chilled 10% glycerol, and lastly 3mL chilled 10% glycerol.
    a. Cells can immediately be used or can be frozen at -80C in 100ul aliquots.
9. Streak out the competent cells to ensure they survived the washes and no contamination.

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Inducing Competence (Modified Salmonella Protocol):

Materials Needed:
Two TSA plates (Bacterial Lawn Grown)
Centrifuge
1.5mL Eppendorf Tubes
50ml Sterile Falcon Tubes
TSB
Pipets
Ice Bucket
One Empty TSA Plate
Magnesium Electroporation Buffer (1mM $MgCl_2$, 1mM HEPES, pH:7)

Protocol (estimated time 2 hours):

1. Remove the bacteria from the plates by pipetting 4mL TSB onto the plates, followed by scraping the cells off of the TSA with an inoculation loop.
2. Once the bacteria are scraped off the plate and floating/suspended in the TSB, angle the plate to one side and pipet up all the TSB (contains the cells)
    a. The solution is then dispensed into a 50mL falcon tube.
3. Repeat steps one and two.

    a. However, place the cells into the same 50ml falcon tube as used in step one and two.
4. Pellet the cells by centrifugation at 2300g for 10min (4C).
    a. Remove the supernatant by pouring it out the top.
        i. Be sure to quickly flame the lid of the falcon tube.
5. Resuspend the bacterial pellet in 20mL ice cold MEB
6. Repeat steps four and five and four again.
7. Resuspend the bacterial pellet in 1ml ice cold MEB (with Glycerol added)
    a. Cells can immediately be used or can be frozen at -80C in 100ul aliquots.
8. Streak out the competent cells to ensure they survived the washes and no contamination.